U.S. National Institute of Health

BULLETIN No. 5 OF THE HYGIENIC LABORATORY,
Treasury Department, U. S. Marine-Hospital Service,
WALTER WYMAN, *Surgeon-General.*

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AN INVESTIGATION

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PATHOGENIC MICROBE

(*B. typhi murium—Danyz*)

APPLIED TO THE

DESTRUCTION OF RATS.

M. J. ROSENAU,

PASSED ASSISTANT SURGEON, DIRECTOR HYGIENIC LABORATORY,
U. S. MARINE-HOSPITAL SERVICE,
WASHINGTON, D. C.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
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AN INVESTIGATION OF A PATHOGENIC MICROBE

(B. typhi murium—Danyz)

APPLIED TO THE DESTRUCTION OF RATS.

[M. J. Rosenau, Passed Assistant Surgeon and Director of the Hygienic Laboratory, United States Marine-Hospital Service.]

The subject of the destruction of rats has assumed great importance within recent times on account of the spread of plague to the four quarters of the globe.

In the short time since 1894, when plague broke from its Eastern confines where it had slumbered so many years, a great mass of exact scientific knowledge has been gathered concerning the disease. The relation of the rat to the spread of the disease has especially engaged the attention of investigators.

During the great outbreaks of plague in the middle ages the unusual mortality among the rats was noticed, and it was obvious then that these animals played some rôle in the spread of the disease. We now know definitely that the rat is susceptible to plague. This rodent sickens and dies of the disease in much the same manner as man. An epidemic may be foretold by a great increase of mortality in rats, for when this occurs it may be shown that the rats first contract the disease and afterwards transmit it to man.

The destruction of rats has, therefore, become of the first importance from the standpoint of the public health and the prevention of the spread of plague.

Plague has threatened our country from both its seacoasts, and it is probable that the only reason it has not spread in San Francisco is due to the fact that the disease has not been prevalent among the rats.

In the spring of 1900, J. Danyz described a new method for the destruction of rats by means of cultures of a certain bacillus (*a*). This bacillus he obtained from a spontaneous epidemic among harvest mice and by means of rather complicated and artificial methods he managed to increase its virulence so that it became pathogenic for the several species of rats. The claim is made that the employment of the cultures

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a Annales de l'Institut Pasteur, April, 1900. A translation of the article by Danyz appeared in the PUBLIC HEALTH REPORTS of May 25, 1900, Vol. XV, No. 21.

of this bacillus, sold under the name of "Rat Virus," is efficient for the destruction of these rodents.

The following work was undertaken in order to test the validity of this claim.

THE ORIGIN OF THE VIRUS.

In 1889 Loeffler discovered the *bacillus typhi murium*, which he isolated from a spontaneous epidemic among white mice, and which he applied with success to the destruction of harvest mice (*m. arvicola*). Other bacteriologists have observed similar epidemics and have isolated the microbes thereof, morphologically identical with the bacillus of Loeffler, but more or less virulent for the several genera and species of the little rodent.

For example : The *B. typhi murium* of Loeffler was only frankly pathogenic for mice (*m. musculus*) and for harvest mice (*m. arvicola*). A bacillus isolated by Laser was pathogenic for the *m. agrarius*, that isolated by Merechkowski for the *spermophiles*, and finally that isolated by Issatchenko for white rats.

Each of these various bacilli is of such variable virulence that it can not be used practically for the destruction of all species of these rodents.

Danyz, therefore, conceived the notion that it would be of great interest, first to extend the field of action of one of these organisms by increasing its virulence so that it would attack other species of rodents and then, this virulence increased, to maintain it at its highest point. This is how he proceeded to solve this problem.

He first isolated a bacillus from a spontaneous epidemic among harvest mice. This organism was a cocco-bacillus, presenting in general the characteristics of the colon bacillus, and resembling the bacillus of Loeffler—*B. typhi murium.* From the first this bacillus showed a slight pathogenicity for gray rats (*m. decumanus*). Out of 10 animals fed with a culture of this microbe 2 or 3 would die; several others would sicken and recover; others still appeared completely refractory. The fact that a certain number of the rats fed with these cultures always succumbed led to the hope that it would be possible to increase the virulence of this particular microbe by the generally accepted methods—that is to say, by a certain number of passages from rat to rat.

Danyz first tried to increase the virulence of the organism by this means, but he found that successive passages from rat to rat, whether by feeding or by subcutaneous injection, ended by enfeebling rather than increasing the virulence of the microbe. He found that it was rarely possible to go beyond 10 to 12 passages. Sometimes the series was stopped at the fifth passage, or even sooner, by the survival of all the animals undergoing experiment. The result was exactly the same, if, instead of alternating each passage through the animal by a culture in bouillon or agar, the bodies of animals dead of a preceding passage were fed to others.

It was, therefore, plain that in the evolution of an epidemic caused by this microbe it was necessary to take account of the indisputable diminution of the virulence of the microbe, as well as the natural resistance of the survivors.

Finally, passages of cultures in collodion sacks, inclosed in the peritoneal cavities of rats, were tried, both in interrupted series and by alternating each sack culture with a culture in bouillon or on agar, but the end was invariably a notable diminution of virulence when administered by the digestive tract.

Danyz finally managed to increase the virulence of the organism, so that it was pathogenic for rats, by the following process:

DANYZ'S METHOD FOR INCREASING AND MAINTAINING THE VIRULENCE OF THE VIRUS.

A culture of the bacillus was selected that was fatal for mice in four to five days, and grown in bouillon to accustom it to an anaërobic existence. This was accomplished by growing the culture in flasks as completely filled as possible. The flasks were placed in the incubator until the culture developed, and then kept at ordinary temperature until a deposit formed and the bouillon became perfectly clear. This may take four or five days, and its object is, as above stated, to accustom the microbe to an anaërobic existence.

From the flasks the culture was passed in a collodion sack, which is kept from twenty-four to thirty-six hours in the abdominal cavity of a rat, and then planted anew in ordinary bouillon and thence again into flasks. The culture was transferred from these last flasks to agar, and it is these cultures on agar that Danyz gave to mice to eat, after having diluted them with water and soaked bread and grain in the dilution.

This series of operations was repeated several times, and at the fourth or fifth repetition a decided increase in virulence was noted. Mice, which died only at the end of four to seven days, now died in thirty-six to sixty hours after the ingestion.

After this decided increase in the virulence for mice was obtained, the mice were replaced by white rats, commencing with young rats a month or six weeks old and, as the passages are continued, taking older rats. Proceeding thus and making collodion sack cultures in the abdominal cavity of the species of the animal which it was desired to infect, the culture was specialized, so that it was rendered sufficiently virulent in ten passages. Operating in this manner, Danyz finally succeeded in rendering regularly virulent for gray rats (*m. decumanus*), then for black rats (*m. ratus*), and finally for white rats, a culture which was originally but slightly virulent for the gray rat and entirely innocuous for the other two.

The bouillon used was made from horse meat with 1 per cent peptone, and to which was added a little carbonate of lime to neutralize the acids which are formed during culture and which rapidly diminish the virulence of the microbe.

After this long and painstaking procedure, Danyz obtained a very virulent culture that, contained in flasks and kept from the influence of light and air, preserved its virulence for several months. Planted on agar it preserved its virulence without appreciable diminution for two months. In bouillon, in flasks or tubes stoppered with cotton, it altered very rapidly.

One can not read the foregoing account without admiration for the patience and perseverance required to increase the virulence of an organism by so many passages through the peritoneal cavities of rats by means of the collodion sack method.

DESCRIPTION OF THE ORGANISM.

The cultures with which I did the following work were obtained from the Pasteur Institute.

The organism is a cocco-bacillus, showing distinct motility. Stains well by the ordinary stains and does not stain by Gram's method.

It grows well at ordinary room temperature; also in the incubator, and on all the ordinary media. In bouillon it produces a uniform cloudiness in twenty-four hours. A slight scum forms after several days' growth, which falls to the bottom when shaken. In Dunham's solution it grows well, but produces no indol in twenty-four hours' growth.

It turns blue litmus milk red, but does not cause coagulation.

It grows the whole length of the stab in gelatin, forming small whitish colonies in the deeper portions of the tube. It does not grow over the entire surface of the gelatin tubes. Does not liquify.

It grows under anaërobic conditions.

It ferments glucose bouillon, but not lactose bouillon.

In glucose bouillon it produces 1–CO_2, 5–H.

It also produces H_2S.

From a general biological standpoint it is plain that this bacillus belongs to the para colon group, and is very similar to the bacillus of rabbit septicæmia, hog cholera, and icteroides—as far as its morphological and cultural characteristics are concerned.

VIRULENCE OF THE VIRUS FOR RATS.

One hundred and fifteen rats were fed with the cultures in various ways during the course of my experiments with the virus. Of these, 46 died—less than half.

Most of the rats used were the gray rat (*m. decumanus*) and the tame white rat. A few (8) of the wild brown, or Norway rat, were used.

The virus is in reality pathogenic for these three kinds of rats when ingested. No special difference was noted in its effects upon the various species.

As the work progressed it soon became evident to me that the result depended largely upon the amount of the culture ingested. By starving rats for a day or two and then giving them all they could be induced to eat and drink of the cultures, a very positive result was obtained. In one instance of 27 rats so fed, all died within a week. If the rats are given a small amount the effect is uncertain—only a few die. In one instance I fed 70 rats with 4 agar tubes, and only 7 died. Upon refeeding them with very large quantities, 9 more died. The survivors were then fed with all they could be induced to eat every day for a week, without effect.

It, therefore, seems plain that a large primary dose proves fatal, and a small dose is not only uncertain, but produces an immunity. This is a very important factor, for it is likely that in the wild state rats would often partake of an amount too small to cause death. Such rats may then subsequently eat large amounts of the culture with impunity.

It would seem, then, that after all, the virus is not so different from the laying out of a chemical poison, depending as it does for its effect upon the amount ingested. A chemical poison, however, does not possess the disadvantage of producing an immunity. Another disadvantage possessed by the virus is the rapid deterioration in virulence which occurs when it is exposed to the action of air and light, or when it becomes dry, as is very apt to happen when laid out for rats in the wild state.

Against this the virus has the very decided advantage over the usual chemical agents used to kill rats that in so far as known it is harmless for man and domestic animals.

THE DISEASE PRODUCED BY THE VIRUS HAS FEEBLE POWERS OF PROPAGATING ITSELF AMONG RATS.

Danyz has pointed out the fact that the epidemic produced by his virus in rats is self limiting. This is due to two causes:

1. The attenuation of the virus.
2. The resistance of the rats.

That the disease can be spread from rat to rat by eating each other can not be doubted, but this method of the spread of the infection is very feeble. I fed rats upon the organs (spleen, liver, etc.), of other rats dead of the infection, without producing any effect. Rats will sometimes eat each other, but sometimes they refuse. I fed dead and sick rats to 10 healthy rats in a cage, and although food was withheld for four days they refused to touch the dead bodies. On other occasions the rats were very vicious, fighting and killing the sick and feeble and eating the dead bodies.

As will be seen from the details of the work which follow, I failed to propagate the disease from rat to rat.

This feeble power of the infection to spread from rat to rat is a decided limitation to the virus as far as its practical application is concerned. It is easy enough to make the rats in a cage eat the cultures soaked in food, but in a wild state this would not be so easy, and if only the rats that eat the virus die, there is little advantage over phosphorous, arsenic, and other chemical poisons.

It is not considered necessary to give in full detail all the experiments made with the virus. A few of the more striking ones follow.

NO. 1. SEVENTY RATS FED ON CULTURES IN INCREASING AMOUNTS.

Seventy white and gray rats were fed April 8 with 4 agar slants (original) of Danyz's virus No. 2, obtained direct from the Pasteur Institute. The cultures were shaken up with water and soaked on dry bread, according to the instructions. The agar itself was also fed to the rats. They ate it all up greedily, food having been withheld for a day previously—

	Died.
Twelve days after feeding	1
Fourteen days after feeding	2
Thirty days after feeding	1
Thirty-one days after feeding	1
Thirty-two days after feeding	1
Thirty-three days after feeding	1
Total	7

Seven of the 70 rats died. One of the 7 died of a peritonitis, due to a long nonmotile bacillus which had no resemblance to the organism that was fed to the rats. The others died of a disease evidently due to the ingestion of the cultures.

In most of the animals the small intestines were found to be the seat of inflammation with enlargement of the lymphatic glands. The spleen and liver showed little change. The lungs were often the seat of congestion and hemorrhagic inflammation—sometimes infarct. The ingestion of the virus causes a local reaction in the intestines, followed by an invasion of the organism into the blood, producing a septicæmia.

The surviving rats, remaining perfectly well to all appearances, were again fed with a fresh culture of the virus. They were now divided into 4 lots and fed with variable amounts in order to determine whether the small quantity ingested produced an immunity and whether the effect is proportionate to the amount eaten.

THIRTY-THREE RATS REFED WITH LARGE QUANTITIES OF BOUILLON CULTURES.

On May 16, after two days' starving, 33 of the surviving rats of experiment No. 1 were given a liter of a young bouillon culture. One-half of this was given as drink, the other half soaked on dry bread. The rats ate and drank most of it greedily. On May 17 and May 18 this was repeated. No other food was allowed; so that in all the 33 rats ingested almost 3 liters of the virus in three days.

	Died.
May 20, four days after feeding	4
May 21, five days after feeding	4
May 22, six days after feeding	1
Total	9

Nine of the 33 rats died. Death came rather suddenly. The others remained apparently healthy. An increased desire to drink probably indicated a feverish condition in some of them. Ordinarily rats die rather slowly from the effects of this virus. They have fever, show symptoms of distress, they are weak and prostrated, and refuse food for a day or two before death.

It, therefore, seems plain that the ingestion of a small amount of the virus killed a few of the susceptible rats—7 out of 70, and left the survivors with a considerable protection, for, after feeding them for three days with overpowering doses, only 9 died out of 33.

TEN RATS REFED ON 10 C. C. OF BOUILLON CULTURE.

Ten of the rats that resisted experiment No. 1 were separated in a cage and subsequently fed with 10 c. c. of a young bouillon culture soaked on bread. This produced no effect.

TEN RATS REFED ON 2 AGAR SLANTS.

Ten of the rats that resisted experiment No. 1 were subsequently fed with 2 agar slants, shaken in water and soaked on bread in accordance with instructions of Danyz. This produced no effect.

TEN RATS GIVEN DEAD RATS TO EAT.

Ten of the rats that resisted experiment No. 1 were placed in a separate cage and deprived of all food for four days, during which time rats sick and dead of the infection were placed in the same cage. They refused to touch the dead bodies. None of the 10 rats took the disease.

It is evident from the foregoing experiments that small primary doses are uncertain in their effects.

Another test was made, using large overpowering doses from the start.

No. 2. TWENTY-SEVEN RATS FED ON VERY LARGE QUANTITIES OF CULTURE.

Twenty-seven white and gray rats were deprived of food for twenty-four hours and then given (April 11) a liter of a young bouillon culture of the virus soaked on bread. In addition they were given another liter of the culture as drink, and 6 agar slants. They ate most of the bread during the night, and drank a considerable quantity of the liquid. Two days following (April 13) they were given another liter of a young culture soaked on bread. On this day the rats seemed sick, 1 died. They ate little or none of the bread, but licked up all the liquid. On April 14, the third day, the rats were sick, very thirsty, refused food, 2 were dead. From this on, the rats refused food and succumbed one after another until all were dead—

	Died.
Two days after feeding	1
Three days after feeding	2
Four days after feeding	10
Five days after feeding	7
Six days after feeding	7
Total	27

All these 27 rats, then, died within a week after being fed with an overpowering amount of the virus.

The result, therefore, seems to depend on the amount ingested as well as the virulence of the cultures.

RATS AND MICE FED ON TOXINS.

A toxin was made by growing the organism in flasks containing 1 per cent peptone bouillon. The flasks were left over night in the incubator at 37° C., and then the growth continued at room temperature. At the end of two weeks the cultures were killed by exposing the flasks to 70° C. for several hours. The resulting product was fed to mice and rats, and was found to produce a certain toxic effect. It seems very probable that the toxin favors the pathogenic action of the organism. This question, not having a direct practical bearing on this particular subject, will be discussed at another time. It may be stated here, however, that animals fed on the toxin seem to acquire a certain amount of immunity against the virulent cultures.

VARIOUS METHODS OF FEEDING.

Rats were fed upon the organs (livers and spleens) of other rats dead of the infection, and in other ways, without producing any effect.

THE SURVIVORS FED 'ON LARGE QUANTITIES.

Finally, I had about 60 rats, which had survived the various experiments, on my hands. I fed them all with large amounts of cultures in order to get rid of them. They were given as much of the cultures as they could be induced to eat, every day for a week, without apparent effect. The immunity produced is, therefore, very positive and lasts at least several months.

SUMMARY.

The substance known as Danyz's Virus consists of a culture of a bacillus belonging to the para colon group. It appears to be indentical with the *bacillus typhi murium* of Loeffler.

This organism is naturally pathogenic for mice, in which rodents it sometimes produces spontaneous epizootics. Its virulence has been raised and specialized by artificial means in the laboratory, so that it has become fatal for rats by ingestion. This artificial virulence is not very stable. It may be maintained under special conditions a few months, but the virulence is apt to fall off, especially on exposure to light and air.

As far as rats are concerned, the effect depends somewhat on the amount ingested. Large amounts are frankly fatal. Small quantities are uncertain. Rats that survive the ingestion of the virus are rendered immune. Such rats may eat large amounts of the virulent virus with impunity.

The infection caused in rats by eating the virus has feeble power of propagating itself from rat to rat. It, therefore, can not produce a widespread epizootic among these rodents. In practical use it must be spread around so that as many of the rats as possible will eat it.

In many respects it resembles a chemical poison, with this great advantage, viz, that it is harmless, in so far as known, to man and domestic animals. It has the great disadvantage, that chemical poisons do not possess, of rendering the animals immune by the ingestion of amounts that are insufficient to kill, or by the ingestion of cultures that have lost a little of their virulence.

In my experiments I succeeded in killing less than half the number (46 out of 115) of rats fed. The conditions in a cage are so much more favorable for the fatal action of the virus than could possibly be the case in nature that it is safe to assert that a less number would succumb in a wild state.

The virus may, therefore, be used as one of the means in the fight against rats, but it is far from being a sure means of exterminating these rodents in a particular place.